

Appendix C  
Manganese Removal Papers

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- b. M.A.T.S.

# BIOREMEDIATION BY MIXED MICROBIAL MATS

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Microbial mats. Microbial mats are natural heterotrophic and autotrophic communities dominated by cyanobacteria (blue-green algae). They are self-organized laminated structures annealed tightly together by slimy secretions from various microbial components. The surface slime of the mats effectively immobilizes the ecosystem to a variety of substrates, thereby stabilizing the most efficient internal microbial structure. Since mats are both nitrogen-fixing and photosynthetic (Paerl et al., 1989), they are self sufficient, solar-driven ecosystems with few growth requirements.

Microbial mats can be generated rapidly by enriching a water surface with ensiled grass clippings (silage). These constructed mats (CM) are durable, tolerant to a variety of toxins and resilient under changing environmental conditions. Additionally, the CM can easily be designed for specific remediation tasks by culturing desired microbes with the cyanobacteria/silage mixture. For example, mats with surface white rot fungus can be produced by this co-culture. Both components (algae and fungus) become tightly integrated and grow rapidly, as a leathery unit, over the surface of the water.

Removal of metals and metalloids by constructed mats. Mats, constructed with specific microbial components, have been developed for various bioremediation applications (Bender and Phillips, 1994). Mats have been found to reduce selenate to elemental selenium (Bender et al., 1991a), remove Pb, Cd, Cu, Zn, Co, Cr, Fe, U and Mn from water (Bender, 1992a; Bender 1991b; Bender et al., 1994a) and to remove Pb from sediments (Bender et al, 1989a). Uranium (0.1 mg/L of U<sup>238</sup>, spiked in groundwater samples) was removed in 3 h. Table 1 presents a summary of metal removal from water and sediments.

Organic degradation by constructed mats. Degradation of recalcitrant organic contaminants has been observed under both dark and light conditions (Bender and Phillips, 1994). The following contaminants have been degraded in water and/or soil media by constructed mats: TNT (Mondecar et al., 1994), chrysene, naphthalene, hexadecane, phenanthrene (Phillips et al., 1994), PCB (Bender, 1993), TCE (Phillips and Bender, 1993, unpublished) and the pesticides, chlordane (Bender et al., 1994b), carbofuran and paraquat (Murray, 1994, unpublished; Phillips and Bender, 1993, unpublished). Radio-labeled experiments with mat-treated carbofuran, petroleum distillates and TCE show that these three compounds are mineralized by mats and mat products, such as biofilms and biofloculents (Phillips and Bender, 1993, unpublished). Recently, in collaboration with the Institute of Paper Science and Technology, we have achieved a 50% reduction (eight-day laboratory treatment) in absorbable chlorinated organics in pulp and paper mill effluent waters. A summary of organic degradation is presented in Table 2.

Treatment of mixed contaminants. Recent data confirms that the mats effectively treat mixtures of organics and heavy metal. Mats simultaneously sequestered Zn and mineralized TCE and chrysene (Phillips and Rodriguez-Eaton, 1993, personal communication).

Immobilized mats. Mats produce slimy secretions which stabilize the members of the microbial community in their stratified structure and also adhere the entire community to a variety of substrates (clay, concrete, activated charcoal and glass wool). Several of these immobilized systems have been

effective in the bioremediation applications: (1) mat/glass wool in columns removed 6.3 g of Zn/Mn per  $M^2$  per d (Bender et al., in press a) (2) mat/glass wool floaters removed 18.6 g Zn/Mn per  $M^2$  per d (Vatcharapijarn, et al., 1994), (3) mat immobilized on clay mineralized carbofuran, chrysene and TCE (57% mineralization in 8 days with 48,000 dpm TCE applied directly to the mat, Phillips, 1993, personal communication).

**Remediation mechanisms.** Constructed mats offer a broad range of mechanisms related to the sequestering of heavy metals, the biodegradation of recalcitrant organics and remediation of mixed organic/inorganic contaminants such as TCE and carbofuran with heavy metals. The diverse microbial components within the mat define the range of molecular, cellular and communal mechanisms available in this ecosystem and likely account for the broad range of successful bioremediation applications demonstrated with this system. Diverse microbes organize into discrete microzones of highly contrasting oxic/anoxic character. These zones exist in close proximity and support aerobic/anaerobic communities simultaneously (Canfield and Des Marais, 1991), thereby offering a unique array of biochemical mechanisms for degradation of recalcitrant organics, such as chlorinated aromatics (Bender et al., 1994b).

Motile bacteria, associated with a mat growing on a soil surface, can penetrate clay soils and have been shown to degrade chlordane mixed within soil phase (Bender et al., 1994b; Murray, 1994, unpublished). Because heavy metal and metalloid contaminants are taken up and concentrated by mats in quiescent ponds (Vatcharapijarn et al., 1994; Bender et al., 1991a), the mechanism of metal transport through the water phase becomes an important question. Scanning electron microscopy/microanalysis research, correlated with chemotaxis studies of the motile bacteria suggest that these microbes become bonded to the metals, and migrate to the mat by responding chemotactically to the cyanobacteria and silage components. (Bender et al., 1989a,b).

Biofloculents likely play a key role in both metal sequestering and organic degradation. It has been found that the biofloculents, released by the mat, bind to heavy metals (Bender et al., in press b; Rodriguez et al., 1994) and also mineralize TCE and the pesticide, carbofuran (Phillips and Bender, 1993, unpublished). Additionally, the cell-free biofilm produced by the mats physically sequesters materials from the sediment region. An insoluble chlordane globule (7 mm dia; 2100 mg/L application level) was picked up by the biofilm, transported to the mat and degraded in 61 days (Bender et al., 1994b). Excised sections of the cell-free biofilm was recently demonstrated to mineralize TCE and carbofuran (Phillips and Bender, 1993, unpublished).

#### Mine Drainage Treatment.

**Design.** Acid coal mine drainage was delivered through a limestone, anoxic drain into an oxidation pond for removal of Fe. Three ponds (1 biological treatment pond, BTP, and 2 controls) 32-44  $m^2$ , were constructed for final removal of Mn and residual Fe. These ponds, receiving drainage from the oxidation pond, were lined with PVC and layered with limestone rocks or pea gravel. A floating mat (1-2 cm thick), composed of filamentous green algae and cyanobacteria was developed in the BTP by enriching with ensiled grass clippings and microbial inocula (initially selected from the site). A second mat formed on the limestone at the pond bottom. Although mats were absent from control ponds, a thin layer (< 0.5 mm) formed over the rocks in these ponds.

**Results.** BTP removed 2.5  $g/m^2/d$  of Mn (Phillips et al., 1994). This removal rate was achieved within the first 2 m from the influent point and continued for approximately a year until the pond was drained. Although there was some Mn-cell binding, metals were primarily deposited as precipitates at the pond bottom. Unlike the control ponds, there was no evidence of metal release from the mat pond. Day/night and winter/summer metal removals were essentially the same. Fe entered the treatment pond primarily as a flocculated precipitate which became entrapped in the filamentous algae. Control ponds showed Mn breakthrough (Mn outflow releases > EPA regulations of 2 mg/L) during nighttime sampling or when mine drainage flow exceeded 4.5 L/min.

**Mechanisms.** Although the conditions of high oxygen and high Eh generated by the field pond mats may be central to the deposit of Mn oxides, other factors may be functional as well. Flocculents were

identified in the water column under the mat. Laboratory research showed that specific biofloculents were released by the mat in response to the presence of  $Mn^{+2}$  (Rodriguez-Eaton et al. 1994). These materials carried surface charges ranging from -58.8 to -65.7 mV. The charges changed to +1.8 in the presence of divalent metal, indicating metal-binding to the biofloculent.

No soil was layered in the pond, so the predictable microbial ecology characterizing the sediment region may not be present in this system. The primary mechanisms of deposit likely were determined primarily by the chemical/biological processes mediated by the mat.

Patent. The Silage-Microbial Mat Construction and Method mentioned herein was the basis for a series of claims for patent protection before the US Patent and Trademark Office in March 1993. In January 1994, notification was received that all claims made by Bender and Phillips were accepted. A patent is being issued to Bender and Phillips.

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**TABLE 1. Metal, metalloid and radionuclide removal in quiescent laboratory ponds.**

Static laboratory ponds	Initial concentration, mg/L	Removal rate, mg metal/m <sup>2</sup> mat/h
Free floating mats	Pb: 117 Se: 37 U <sup>238</sup> : 0.12	129 6 3.19
Mat immobilized on glass wool layered in baffled tanks	Mix of Cr: 24 Co: 24	10,129 10,052
Mat immobilized on floaters	Mix of: Zn: 22 Mn: 18	313 462
Excised mats	Mix of: Cu: 284 Zn: 3,021 Cd: 19	378 3,778 356
Field pilot: acid mine drainage	Initial concentration, mg/L  Mn: 3.3 - 6.5	Removal rate, mg metal/m <sup>2</sup> mat/d  2.59

**Free floating mat.** Self-buoyant mats were cultured on the surface of laboratory ponds containing Pb or Se. Initial solution of selenate was reduced in part to elemental selenium which deposited in the surface mat (Bender et al. 1991a). Pb was deposited in the mat as PbS (Bender et al. 1989). The pH for the free floating mats was 6 to 8.

**Mat immobilized on floaters.** The mat was attached to glass wool balls that were floated in Zn/Mn-contaminated water at pH 7 to 9 (Bender, 1992b).

**Excised mats.** Small sections of mat were excised and applied to a mixed solution of Cu, Zn, Cd, and Fe sample from Iron Mountain Mine drainage in California (Bender et al. 1991b). The pH was adjusted to 3 to 4 before adding mat sections.

**Microbial/green algae mat.** A floating mat (1-2 cm thick), composed of filamentous green algae and cyanobacteria was developed by enriching with ensiled grass clippings and microbial inocula (initially selected from the site). A second mat formed on the limestone at the pond bottom.

TABLE 2. Biodegradation of contaminants by microbial mats.

Contaminant	Concentrations, mg/L		Time and % degradation	Mineralization
	Initial	Final		
2,4,6-trinitrotoluene (TNT)	100	<1	>99% in 6 days	
2,4-Dinitrotoluene (DNT)	217	26	88% in 4 days	NP
Chlordane in water	2,100	61	97% in 35 days	+
in soil	200	146	27% in 25 days	
Petroleum distillates <sup>(1)</sup>				
hexadecane	768	697	9% in 90 days	+
phenanthrene	374	284	24% in 90 days	+
chrysene	157	125	20% in 90 days	
PCB (3 Cl)	50	40	20% in 15 days	
(6 Cl)	100	63	37% in 33 days	
Pulp & paper mill Chlorinated organics	26	11	66% in 1 day	NP
Color reduction			39% in 1 day	
<u>Soil mix of:</u>				
Paraquat	50	29	42% in 21 days	NP
+				
Carbofuran	50	20	60% in 21 days	+
<u>Mixed organic + inorganic:</u>				
TCE + Zn	Simultaneous mineralization of TCE			+
Chrysene + Zn	and chrysene with Zn sequester			+

**Experimental.** Mineralization experiments were performed with  $C^{14}$ -labeled substrate; detection of entrapped labeled carbon dioxide determined percent mineralization. Occurrence of metabolic products in the water column and mat matrix confirmed that the process was degradation rather than simple adsorption. Soil/chlordane and pulp and paper mill experiments are in progress; no attempt has been made to identify metabolic products in these experiments. NP = mineralization experiments were not performed. In mixed contaminants no inhibition of processes was observed. The rates of organic degradation and metal sequestering were essentially the same in mixture as they were in single contaminant exposure.

**Credibility of data results:** Experiments were performed in three triplicate trials except in cases where radio-labeled materials prevented this number. In such cases single experiments were performed with triplicate trials (reaction flasks). In metal-sequestering experiments, mass balances fell within acceptable limits ( $\pm 1\%$ ). Every sample set included a calibration of the instrument. In chlordane-degradation experiments, parallel analyses were performed by two commercial laboratories. Results verified that 10 mg chlordane applied below the mat was reduced to <1 mg in 5-7 days.

**References:** TNT = Mondecar et al., 1994; Chlordane = Bender et al., 1994; Petroleum distillates = Phillips et al., 1994; PCB = Bender, 1993; TCE, chrysene, carbofuran, paraquat = Phillips and Bender, 1993, Report to Pacific Northwest Laboratories Battelle.



# POTENTIAL FOR BIOREMEDIATING USING CONSTRUCTED MIXED MICROBIAL MATS

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## ABSTRACT

Microbial mats are natural heterotrophic and autotrophic communities dominated by cyanobacteria (blue-green algae). They are self-organized laminated structures annealed tightly together by slimy secretions from various microbial components. The surface slime of the mats effectively immobilizes the ecosystem to a variety of substrates, thereby stabilizing the most efficient internal microbial structure. Constructed microbial mats can be generated rapidly by enriching a water surface with ensiled grass clippings. These constructed mats are durable, tolerant to a variety of toxins and resilient under changing environmental conditions. The mats can be designed for specific tasks by inoculating the cyanobacteria/silage with selected microorganisms. Mats constructed with specific microbial components have been developed for various bioremediation applications: removal of metals, organic degradation, treatment of mixed contaminants, biological treatment ponds, and soil remediation. Constructed mats offer a broad range of mechanisms related to the sequestration of heavy metals, the biodegradation of recalcitrant organic compounds, and the remediation of mixed organic/inorganic contaminants such as TCE and

carbofuran with heavy metals. Diverse microbes organize into discrete microzones of highly contrasting oxic/anoxic character. These zones exist in close proximity and support aerobic/anaerobic communities simultaneously, thereby offering a unique array of biochemical mechanisms for degradation of recalcitrant organics such as chlorinated aromatic compounds. Because heavy metal and metalloid contaminants are taken up and concentrated by mats in quiescent ponds, the mechanism of metal transport through the water phase becomes an important question. Studies of the chemotaxis of the motile bacterial suggest that these microbes become bonded to the metals and migrate to the mat by responding chemotactically to the cyanobacteria and silage components. Biofloculants produced by the mats likely play a key role in both metal sequestering and organic degradation. It has been found that the biofloculants released by the mats bind to heavy metals. Additionally, the cell-free biofilm produced by the mats physically sequesters materials from the sediment region. Motile bacteria, associated with a mat growing on the surface of a clay soil, have been shown to degrade chlordane mixed within the soil phase. Biological treatment ponds using the constructed mats were developed

for treating acid coal mine drainage. The biological treatment ponds were operated for a year to remove Fe and Mn from the mine drainage. In laboratory studies, it was observed that specific bioflocculants were produced by the mats in response to the metals. The diverse microbial components within the mat define the range of molecular, cellular, and communal mechanisms available in this ecosystem and likely account for the broad range of successful bioremediation applications demonstrated with this system.

## INTRODUCTION

### *Microbial mats*

Microbial mats are natural heterotrophic and autotrophic communities dominated by cyanobacteria (blue-green algae). They are self-organized laminated structures annealed tightly together by slimy secretions from various microbial components. The surface slime of the mats effectively immobilizes the ecosystem to a variety of substrates, thereby stabilizing the most efficient internal microbial structure. Since mats are both nitrogen-fixing and photosynthetic (1), they are self-sufficient, solar-driven ecosystems with few growth requirements.

Microbial mats can be generated rapidly by enriching a water surface with ensiled grass clippings (silage). These constructed mats (CM) are durable, tolerant to a variety of toxins and resilient under changing environmental conditions. Additionally, the CM can easily be designed for specific remediation tasks by culturing desired microbes with the cyanobacteria/silage mixture. For example, mats with surface white rot fungus can be produced by this co-culture. Both components (algae and fungus) become tightly integrated and grow rapidly, as a leathery unit, over the surface of the water.

### *Patent*

The microbial mat treated herein was the basis for a series of claims by Bender and Phillips for patent protection before the US Patent and Trademark Office in March 1993. In January 1994, notification was received that all claims made were accepted for the patent.

### *Removal of metals, metalloids, organic contaminants and mixed wastes by constructed constructed mats*

Mats produce slimy secretions which stabilize the members of the microbial community in their stratified structure and also adhere the entire community to a variety of substrates (clay, concrete, activated charcoal and glass wool). Mats, constructed with specific microbial components, have been developed for various bioremediation applications (2). Degradation of recalcitrant organic contaminants has been observed under both dark and light conditions in water or soil (Table 1). Mats have been found to remove heavy metals from water and sediment (Tables 2 and 4). In collaboration with the

Institute of Paper Science and Technology, we have achieved a 50% reduction (eight-day laboratory treatment) in absorbed chlorinated organics in pulp and paper mill effluent waters. Recent data confirms that the mats effectively treat mixtures of organics and heavy metals (Table 3).

## MATERIALS AND METHODS

### *Preparation of mats*

Microbial mats are generated by enriching a water surface with ensiled grass clippings (7 g wet wt/L). Inocula of desired microbes are added with the silage. These microbes can be supplied as small sections of mature mats (maintained as desiccated or wet stock supply) or as separate strains of the constituent bacteria (cyanobacteria, purple autotrophic bacteria, and a variety of aerobic/anaerobic heterotrophs). The microbial consortium self-organizes in 5-7 days and forms a leathery sheet around the silage. Depending on the format of the remediation application, mats can be cut and added as excised sections, packed into columns, grown out on soil or sediment, floated on pond surfaces, layered in baffled tanks or broadcast over an inert substrate (i.e., organic mesh or glass wool). The mat grows and attaches to most substrates forming immobilized cell preparations.

### *Application of contaminants and conditions*

Except for samples from the Iron Mountain Mine (IMM), initial pH levels in all experiments were 7-8; pH levels generally rose to 8-9 during the light period, and declined back to 7-8 during dark periods. The IMM samples were initially adjusted to pH 3-4 before adding the excised sections of mat. Metal precipitates, resulting from the pH elevation, were removed by settling before beginning the experiments. Initial concentrations listed in Table 1 represent those levels of soluble metals remaining after this initial metal removal. Except for the field pond experiments, the metal sequestering experiments were performed in continuous light. A 12/12 h light-dark cycle was used for organic degradations, unless otherwise indicated in the data table. Metals were added as soluble nitrates. Organics were generally solubilized in a solvent before adding to mats and water columns. In the case of contaminants which had very low solubility (TNT) or those added in concentrations that exceeded the solubility level (chlordanes) the liquid globules or solid particles were allowed to remain at the bottom of the reaction flask. This posed no problem because contractile biofilms, generated by the mats, actively transported the materials to the surface where they became enmeshed in the mat matrix. In the case of chlordanes/soil experiments, chlordanes was mixed in clay soil. Mats were cultured on the moist soil surface. Experimental controls were either the contaminant placed under experimental conditions without mats or with heat-killed mats.

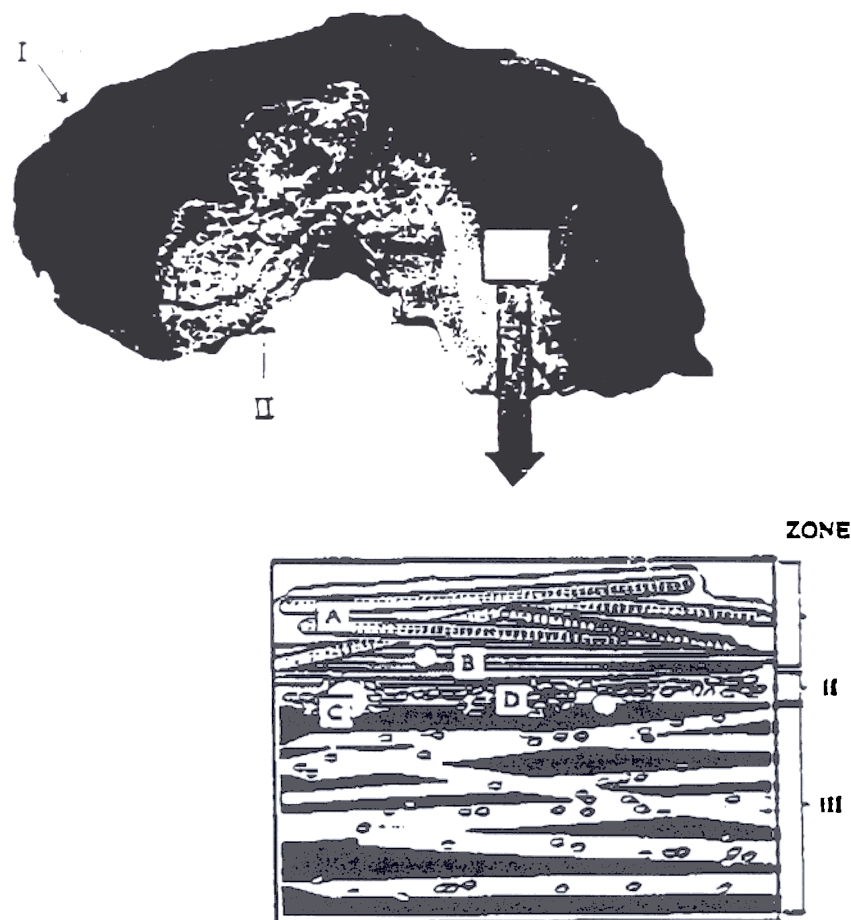


Fig. 1. Upper. Mat immobilized on glass wool floaters showed 70% (wgt/wgt) metal binding on mass and associated organic material. I = mat contains 43 mg metal/g organic. Lower. Schematic of microbial mat attached to ensiled grass clippings. Zones: I = photosynthetic zone with cyanobacteria (A), II = heterotrophic bacteria (D) colonized around ensiled grass (B), III = glass wool with associated bacteria and organic residues released from the surface. Entrapped gases (C) maintain the buoyancy of the mat. Approximate depth for Zones 1, 2 and 3, respectively are 2 mm, 1 mm and 3.5 cm.

In Table 2 the Cr/Co removals show the highest rates using a baffled tank/immobilized mat system. Although the metal removal levels were reduced to <50% by the last 5 flows, the mats remained live and, after an addition of minimal salts medium, continued to grow. The uranium removal data in Table 2 shows that mats not only deal with high levels of inorganic contaminants, but can detect and remove  $\mu\text{g}$  concentrations as well.

Metal analysis of the mat and associated organics from the floating mat experiments show a 70% wt/wt binding of the metals to the mat material. This results in a discrete unit of mat-metal enmeshed in the glass wool floater (Fig. 1). The product might be subsequently vitrified into a concentrated pellet for metal disposal.

#### Field treatment of mine drainage

Acid coal mine drainage in Alabama was delivered into 32-44  $\text{m}^2$  ponds for removal of Mn. A floating mat (1-2 cm thick), composed of filamentous green algae and microbial mat, was developed in one pond. A second mat formed on the limestone at the pond bottom. The pond with an algae mat removed 2.5  $\text{g}/\text{m}^2/\text{d}$  of Mn (9). This removal rate was achieved within the first 2 m from the influent point and continued for approximately a year until the pond was drained. Although there was some Mn-cell binding, metals were primarily deposited as precipitates at the pond bottom. Unlike the control ponds, there was no evidence of metal release from the mat pond. Metal removal was essentially the same during day/night and winter/summer conditions. Control ponds showed Mn breakthrough (Mn outflow releases > EPA

#### *Design of contaminant/mat exposure*

Organic materials were generally added to the water column under the mat in a quiescent system. Only in the case of DNT was slow shaking (50 rpm) applied. Inorganic contaminants were applied in several design formats. Uranium was added to actual groundwater samples taken from the Department of Energy site at Hanford, WA. Chromium and cobalt (in mixed solution) were passed through a baffled tank layered with glass wool with an immobilized mat (each flow: 200 ml of 20 mg/L each metal, passed through baffle in 40 sec). In IMM experiments excised mats (9 cm<sup>2</sup>) were added to quiescent system containing 200 ml sample, diluted 1:1 with silage wash medium. Floating mats (floaters) were prepared with 4 g glass wool containing mats (1-2 mm) immobilized on the surface. These were applied to 1 L of mixed metal solution (18-22 mg/L Zn + Mn).

#### *Field pond experiments*

Microbial mat bound to green algae in a limestone-lined pond was used for manganese removal from coal mine drainage in Alabama from August 1992 to the present. Mine drainage flowed from a seep, through an anoxic drain and into an oxidation pond. Before entering the biological treatment pond, the oxidation pond water flowed through a trickling filter to remove some of the water's iron content. All microorganisms were isolated from the site, cultured in the laboratory and returned to the site for inoculation. Green algae initially established voluntarily. Control ponds included one with only a limestone substrate and another with a pea gravel substrate. Water quality parameters monitored were manganese and iron concentrations, temperature, dissolved oxygen, pH, redox, conductivity and alkalinity. Water flow rates averaged 4.2 L/min.

#### *Sample preparation and analysis*

**Organic degradation experiments.** All methods described below were taken from EPA SW-846 (8). Water columns under mat were extracted by separatory funnel liquid-liquid extraction method #3510. Solids (mats or soil) were extracted by either soxhlet method #3540 or ultrasonic extraction method #3550A. Analysis of residual parent compound and metabolic products were performed as follows: **PCB/chlordane:** GC method #8080A with electron capture detector. Column was Supelcoport 100/120 mesh coated with 1-5% SP-2250/1-95% SP-2401 packed in a 1.8 m x 4 mm ID glass column or equivalent (Supelco, Inc.). Carrier gas of 5% methane/95% argon was used at a flow rate of 60 ml/min and 200° isothermal. **TNT/DNT:** HPLC method #8330 with UV detector, 254 nm. Column was reverse phase Supelcosil-LC-18, 25 cm x 4.6 mm x 5 µm packed with 5 µm spherical silica (Supelco, Inc.). Mobile phase was isocratic at 50% methanol/50% water with flow rate of 1.5 ml/min. Injection volume was 100 µL.

**Metal sequestering experiments.** All samples were digested prior to analysis by a CEM MDS-2000 microwave oven. Liquid samples were digested with trace metal grade nitric acid for a time period of 3 cycles of 10 mins each and a maximum permissible pressure of 80 psi. Solid samples were digested similarly for a 5-cycle period: first 3 cycles of 5 mins each and last 2 cycles of 10 mins each, with a maximum permissible pressure of 80 psi. Digested samples were analyzed for metal concentration with Varian Spectra-20 Atomic Absorption Spectrophotometer.

**Organic mineralization experiments.** Eight organic substrates were tested for the ability of microbial mat to mineralize them to <sup>14</sup>CO<sub>2</sub> (Table 4). Experiments were conducted in a closed quiescent system with mat floating on water. In additional experiments, TCE, chrysene and carbofuran were applied to soil. Mineralization rates were determined by the amount of radioactivity (<sup>14</sup>CO<sub>2</sub>) present in a potassium hydroxide trap. In the case of chlordane and hexachlorobiphenyl (PCB), a <sup>14</sup>C budget determined the amount of <sup>14</sup>CO<sub>2</sub> remaining in the water and re-incorporated into microbial mat via photosynthesis. Thin-layer chromatography was used to separate parent compound and dechlorinated metabolites. Visualization of spots by autoradiography and scintillation counting was used for parent compound and metabolites. TCA precipitation followed by 8-9 washes was the technique used to determine if <sup>14</sup>C was in the protein fraction of the microbial mat.

## **RESULTS AND DISCUSSION**

### *Explanation of experimental results*

Bioremediation results are described in Tables 1, 2, 3 and 4. The microbial mats were effective in removing and/or degrading each class of compounds, including the mixture of TCE and Zn. Metabolic products were observed in all organic degradation experiments. In the case of DNT, eight metabolites were observed over a 25-day treatment. Metabolite identification is in process; 2-Methyl 5-Nitro Aniline has been confirmed. However, six of the eight metabolites disappear over time.

Mineralization experiments showed complete degradation with several recalcitrant compounds including hexachloro-PCB and chlordane. In a three-week treatment C<sup>14</sup>-chlordane, mass balance analysis of the carbon label showed that 17% was recovered as CO<sub>2</sub>, 78% as mat protein and 2% as highly polar metabolites (Bender et al., in preparation). No parent compound was recovered. This complete degradation occurred only in a dark/light cycle (12/12 h). When mat was exposed to chlordane under continuous darkness, significant quantities of parent compound were recovered. The chlordane/soil data is considered to be a preliminary result, until the experiments are confirmed with labeled chlordane.

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**TABLE 1**  
**REMEDIATION OF ORGANIC CONTAMINANTS BY MICROBIAL MATS**

ORGANIC CONTAMINANT <sup>a</sup>	INITIAL CONCENTRATION mg/L or mg/kg	TIME AND % DEGRADATION <sup>b</sup>
2,4,6-trinitrotoluene	100	>99% in 6 days
2,4-Dinitrotoluene	217	88% in 4 days
Chlordane In water In soil	2,100 200	97% in 35 days 27% in 25 days
Hexachlorobiphenyl (PCB)	100	37% in 33 days

Microbial mats were applied as free-floating mats on the surface of contaminated water (except with the soil/chlordane experiment in which mat was applied to soil surface and chlordane was mixed in soil). Where the organic substrate was insoluble in water, contractile biofilms generated by the mat actively sequestered the contaminant globule. (13) (14) (15)

b. Degradation was distinguished from simple absorption by disappearance of parent compound from the mat and water, occurrence of metabolic products and mineralization experiments (the latter is presented in Table 4).

**TABLE 2**  
**REMEDIATION OF INORGANIC CONTAMINANTS BY MICROBIAL MATS**

INORGANIC CONTAMINANT	INITIAL CONCENTRATION, mg/L		REMOVAL RATE mg metal/m <sup>2</sup> mat/h
Free floating mats <sup>a</sup>	U <sup>238</sup>	0.12	3.19
Mat immobilized on glass wool and Layered in baffled tanks	Mix of		10,129 10,052
	Cr	24	
	Co	24	
Mat immobilized on floaters <sup>b</sup>	Zn	22	313
	Mn	18	462
Excised mats applied to Iron Mountain mine sample <sup>c</sup>	Cu	284	378
	Zn	3,021	3,778
	Cd	19	356
Acid mine drainage <sup>d</sup>	Mn	3.3 - 6.5	2.59 mg Mg/m <sup>2</sup> /day

a. **Free floating mat.** Self-buoyant mats were cultured on the surface of laboratory ponds' surface mat. Pb was deposited in the mat as PbS. The pH for the free floating mats was 6 to 8. (17)

b. **Mat immobilized on floaters.** The mat was attached to glass wool balls that were floated in Zn/Mn-contaminated water at pH 7 to 9. (16)

c. **Excised mats.** Small sections of mat were excised and applied to a mixed solution of Cu, Zn, Cd, and Fe sample from Iron Mountain Mine drainage in California, a Superfund site. The pH was adjusted to 3 to 4 before adding mat sections. (5)

d. **Pond treatment of acid mine drainage.** A floating mat (1-2 cm thick), composed of filamentous green algae and cyanobacteria was developed by enriching with ensiled grass clippings and microbial inocula (initially selected from the site) on a 40 m<sup>2</sup> field pond. A second mat formed on the limestone at the pond bottom. (9)

**TABLE 3 REMEDIATION OF MIXED WASTE BY MICROBIAL MATS**

MIX WASTE CONTAMINANT	INITIAL CONCENTRATION, mg/L		REMOVAL/MINERALIZATION <sup>a</sup>	
Free floating mats	Mix of TCE + Zn			
	<sup>14</sup> C-TCE	0.002	TCE	147 µg/kg mat, mineralized
	Zn	20	Zn	264 mg/m <sup>2</sup> /h
	Mix of chrysene + Zn			
	<sup>14</sup> C-chrysene	0.079	Chrysene	59 µg/kg mat, mineralized
	Zn	20	Zn	Not determined

<sup>a</sup> Mineralization experiments were performed with C<sup>14</sup>-labeled TCE or chrysene; detection of entrapped labeled carbon dioxide determined percent mineralization. With mixed contaminants neither inhibition of organic contaminant mineralization nor metal sequester were observed. For example, TCE + Zn mineralized at a rate of 147 µg/kg, whereas TCE alone mineralized at a rate of 119 µg/kg. Chrysene + Zn mineralized at a rate of 59 µg/kg, whereas chrysene alone mineralized at a rate of 64 µg/kg. (18)

**TABLE 4  
MINERALIZATION OF ORGANIC CONTAMINANTS BY MICROBIAL MAT**

ORGANIC CONTAMINANT <sup>a</sup>	INITIAL CONCENTRATION µg/L	TIME AND % DEGRADATION	LIGHT/DARK CONDITIONS
Petroleum distillates			
hexadecane	768	9% in 90 days	dark
phenanthrene	374	24% in 90 days	dark
chrysene	157	21% in 90 days	dark
TNT	<1000 <sup>b</sup>	4% in 45 days	dark
Chlordane	133	13% in 21 days	12L:12D
PCB	133	17% in 21 days	12L:12D
TCE	2	21% in 15 days	dark
Carbofuran	12	4% in 15 days	dark

<sup>a</sup> Microbial mats were applied as free-floating mats on the surface of contaminated water. Mineralization experiments were performed with C<sup>14</sup>-labeled substrate; detection of entrapped labeled carbon dioxide determined percent mineralization. (18) (19)

<sup>b</sup> Exact concentration not available.

regulations of 2 mg/L) during night-time sampling or when mine drainage flow exceeded 4.5 L/min. Although the conditions of high oxygen and high Eh generated by the field pond mats may be central to the deposit of Mn oxides, other factors may be functional as well and are described below.

#### *Possible remediation mechanisms*

Mixed microbial remediation systems have several distinct advantages. Specific detoxification mechanisms unique to all constituent strains of the mat are accessible in the consortium. Thus a broader variety of cellular releases (enzymes, bioflocclents) are available with the mat consortium than with a treatment system dominated by a single microbial strain.

The mat consortium generates micro-zones of unique chemistry characterized by discrete oxic and anoxic zones throughout the microbial matrix (Fig. 1). This phenomenon is amplified by the close association of phototrophs and heterotrophs. Thus, reductive dechlorination can likely occur by anaerobic processes in the anoxic zones, while carbon ring lysis is accomplished by the aerobes in the adjacent oxic zones. The rapid mineralization of chlordane might be explained by this community micro-structure of the mat. Either metabolic process (aerobic or anaerobic) can be amplified in mat by simply changing the light intensity and duration, thereby altering the conditions of Eh, pH and oxygen concentration.

Metal sequestering and interment of metals are likewise controlled both at the cellular/molecular and the community levels. Certain species of cyanobacteria produce negatively charged, flocculating macromolecules, which likely bind to the metals in the water column (10). This provides initial protection to the microbial community contacting the toxic metal. At the community level, the anaerobic zones harbor sulfur reducing bacteria (11), which generate hydrogen sulfide in the anoxic zones. Thus, sulfide is available for metal precipitation in the interstitial spaces of the mat. High concentrations of oxygen, produced during photosynthesis, becomes entrapped in the mat surface slime. Oxygen, therefore, remains available for oxide precipitation over long time periods in the photosynthetic zone of the mat.

The uptake of hexavalent chromium [Cr(VI)] at neutral to alkaline pH values of these studies suggests that the process is, at least to some extent, microbially mediated. This observation is based on several considerations. Hexavalent chromium is, in all of its forms, highly soluble in water and, consequently, unlikely to be precipitated or otherwise bound in high loadings by the microbial solids of the mats. The trivalent form of chromium which is the likely terminal oxidation state of

any reduction of the hexavalent chromium is an excellent cationic form for deposition as solid  $\text{Cr}(\text{OH})_3$ , which has a  $\text{pK}$  of 37. However, conversion of hexavalent chromium to trivalent chromium will not occur at these elevated pH levels even in the presence of high concentrations of organic electron donors (12). In studies on the reduction of hexavalent chromium by *E. coli*, it was found that chromium reduction occurred concurrently with the respiration of organic electron donors such as glucose.

Based on these considerations, the rapid uptake of chromium to very high loadings in these mat studies suggests strongly that the process was mediated by some enzyme or other active reduction catalyst which is elaborated by the mat system as a defense mechanism. The mechanism acts to convert the highly toxic hexavalent chromium to the far less hazardous trivalent form which is precipitated rapidly as the hydroxide at the ambient neutral to alkaline pH levels.

The high uptake of cobalt by these mats systems was likely a consequence of simple precipitation as some sparingly soluble cobalt salt. Obvious candidates as precipitating anions are hydroxide, carbonate and, given the known presence of strongly reducing microzones in the mats, sulfide. At present, there is insufficient data to distinguish among these alternatives. Nonetheless, the mat systems appear to have very high capacities for uptake of cobalt.

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